

Morphological and molecular analysis of thrips (Thysanoptera: Thripidae) diversity on avocado and dandelion: New species records for Colombia and limitations for molecular differentiation of two species

Análisis morfológico y molecular de la diversidad de trips (Thysanoptera: Thripidae) en aguacate y diente de león: Nuevos registros de especies y las limitaciones para diferenciar dos especies

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Abstract: Thrips are important pests of avocados causing damage to the fruits, making them unsuitable for exportation. Many of them are quarantine species and their identification is necessary. Despite their importance, not many studies describe thrips diversity on avocado, especially in Colombia, partly because identification of some of the species is difficult using classical taxonomic keys. In this study, 93 adult thrips specimens were collected on avocado and adventitious dandelion plants from plantations in the department of Antioquia, Colombia. Morphological characters were used to classify 62 of them and all of them were analyzed by PCR amplification and sequencing of cytochrome c oxidase subunit I (COI) gene and internal transcribed spacer (ITS) region. The species identified by morphological features were *Frankliniella gardeniae* Moulton, *F. gossypiana* Hood, *F. panamensis* Hood, *Liothrips perseae* Watson, *Thrips palmi* Karny, *T. simplex* (Morison), *T. trehernei* Priesner, *Microcephalothrips abdominalis* (Crawford DL), and *Scirtothrips hansonii* Mound & Hoddle (Tubulifera), being *F. panamensis*, *M. abdominalis*, and *S. hansonii* reported for the first time on avocado in Colombia. *Thrips simplex* and *T. trehernei* were also found on adventitious dandelion plants, but not on avocado. Phylogenetic analysis of COI and ITS sequences showed that most species, except for *F. gardeniae* and *F. gossypiana* were mainly identified with the COI gene. Furthermore, F_{ST} values obtained between them suggest no genetic differentiation at COI level. The results obtained here contribute to the knowledge of the diversity of thrips species found on the avocado crop in Colombia and are meaningful for pest management.

Keywords: Cytochrome Oxidase subunit I, avocado crop, avocado pest, *Frankliniella*, molecular taxonomy, *Scirtothrips*, *Thrips*.

Resumen: Los trips son plagas de importancia económica en el cultivo de aguacate. Producen daños en los frutos impidiendo la exportación. Muchas de sus especies se consideran cuarentenarias y su identificación es obligatoria. A pesar de su importancia, existen pocos estudios que describan su diversidad, especialmente en los cultivos de aguacate de Colombia, ya que la identificación de algunas de las especies es difícil con claves taxonómicas clásicas. En este estudio, se recolectaron 93 especímenes adultos de trips en cultivos de aguacate y la planta adventicia diente de león como una fuente que aloja trips, en el departamento de Antioquia, noreste de Colombia. Se amplificaron y secuenciaron el gen citocromo oxidasa I (COI) y el espaciador interno transcrito (ITS), y se identificaron morfológicamente 62 de ellos. Las especies clasificadas morfológicamente fueron *Frankliniella gardeniae*, *F. gossypiana*, *T. palmi*, *F. panamensis*, *Liothrips perseae*, *Microcephalothrips abdominalis*, *Scirtothrips hansonii* Mound & Hoddle, *Thrips simplex*, y *T. trehernei*, (Tubulifera), reportándose *M. abdominalis*, *S. hansonii*, y *F. panamensis* por primera vez en aguacate en Colombia. *Thrips simplex* y *T. trehernei* solo se encontraron en diente de león. Los análisis filogenéticos realizados con COI e ITS mostraron concordancia con la taxonomía de todas las especies excepto *F. gardeniae* y *F. gossypiana*. Como consecuencia, los valores de F_{ST} obtenidos entre ellos sugieren que no hay diferenciación genética a nivel de COI. Los resultados obtenidos contribuyen al conocimiento de la diversidad de especies de trips que se encuentran en el cultivo de aguacate en Colombia y son importantes para el manejo de plagas.

Palabras clave: Citocromo Oxidasa I, cultivos de aguacate, *Frankliniella*, plagas de aguacate, *Scirtothrips*, taxonomía molecular, *Thrips*.

Introduction

Colombia registered 39.172 hectares of avocado (*Persea americana* Mill.) harvested in 2017, ranking third worldwide in planted area and fourth in total production with 314.275 Tons (FAO, 2018). In recent years, avocado has been considered one of the most important crops for exportation in this country. The department of Antioquia stands out in Colombian avocado production with 11.321 hectares and an increase of 126 % in planted area in the last 5 years (Ministerio de Agricultura, 2018). However, some factors limit competitiveness such as pests which require constant management, increasing production costs (Bravo-Pérez *et al.* 2018).

Thrips (Thysanoptera) represent serious invader pests of avocado because they produce scars on the fruit, making it unsuitable for export markets (Cambero-Campos *et al.* 2011). They are difficult pests to control due to their small size, cosmopolitan distribution, high reproductive rate, polyphagous habits, invasive behavior (both in greenhouses and in the field), and a limited number of natural enemies (Brunner *et al.* 2002; Monje *et al.* 2012; Moudén *et al.* 2017). For these reasons, many thrips species have been considered quarantine species for many countries. The exaggerated use of pesticides has led to resistance, residue problems on marketable crops, toxicity towards beneficial non-target organisms, and contamination of the environment (Moudén *et al.* 2017).

One important problem is their taxonomical identification at the species level due to their imperceptible morphological differentiation, intraspecific polymorphisms, and sexual dimorphisms (Brunner *et al.* 2004; Tyagi *et al.* 2008; Iftikhar *et al.* 2016). The requirement of an expert in thrips morphology is essential for taxa identification. However, an alternative to morphological identification is the use of molecular techniques, which can be used together with morphological variation to identify species, as well as to improve the taxonomical status and reconstruct phylogenies (Brunner *et al.* 2004; Buckman *et al.* 2013; Iftikhar *et al.* 2016). Several molecular markers, including nuclear and mitochondrial loci, have been used for species identification and phylogenetic analysis (Asokan *et al.* 2007; Buckman *et al.* 2013; Iftikhar *et al.* 2016; Tyagi *et al.* 2017).

Despite their importance, there are not many studies reporting the thrips species affecting avocado and often they are recognized as belonging to a species complex (Echeverri-Florez *et al.* 2004; Cambero-Campos *et al.* 2011). In Colombia, a morphological characterization made from adults collected from avocado leaves and flowers at Caldas and Risaralda departments (West-Central Colombia) allowed the identification of the phytophagous species: *Selenothrips rubrocinctus* Giard, and *Frankliniella gardeniae* Moulton, and individuals of the genera: *Leptothrips* and *Karnyothrips* have predatory habits (Echeverri-Florez *et al.* 2004). A recent study based on the molecular identification of the genera *Arorathrips*, *Caliothrips* *Frankliniella*, *Leptothrips*, *Neohydathrips*, and *Scirtothrips*, was conducted in Mexico using 44 specimens collected from avocado and sequencing of the mitochondrial COI (Cytochrome Oxidase I unit) gene (Bravo-Pérez *et al.* 2018).

Given the little knowledge on thrips species in Colombia and the Antioquia department, the aim of this study was to identify the species of thrips present on avocado trees from commercial plantations in Antioquia. In addition, we tested if the use of molecular techniques could aid in the identification of the species present in this crop easier. For this, 93

specimens were sampled, taxonomically identified by morphological characters, and analyzed by PCR amplification and sequencing of the Cytochrome Oxidase I (COI) gene and Internal transcribed spacer (ITS) region of each specimen. Results obtained here are useful since they widen the information on the thrips species affecting avocado and could be used to improve an integrated pest management strategy based on COI sequencing as a simple and accurate method that facilitates the identification of thrips species (Rebijith *et al.* 2014).

Materials and methods

Specimen's collections. Thrips specimens (adults) were randomly collected from several commercial avocado trees during 2017 in 7 avocado orchards (approx. 1-2 ha area/orchard), in the department of Antioquia in Northeast Colombia (Supplementary Table 1). For molecular analysis, 93 adult individuals were collected from avocado, *P. americana* Mill. (Lauraceae) and dandelion, *Taraxacum officinale* (L.) Weber ex F.H.Wigg. (Asteraceae) plant inflorescences. The dandelion plants were considered in this study because they could be a reservoir of avocado thrips and because they are commonly found in avocado orchards in the region. Thrips were collected and transferred to a 50 mL Falcon tube with ethanol at 96%. Location was recorded with GPS on each sampling site. The tubes were kept at -20 °C at the laboratory of Molecular and Cell Biology at Universidad Nacional de Colombia in Medellín until processing. Of 93 thrips, morphological identification was carried out on 62 specimens based on various characters described by Mound and Marullo (1996). For taxonomical identification, the specimens were sent to Dr. Laurence Mound in Australia at the CSIRO Institute, Canberra, and followed the classification of Mound and Hoddle (2016). Species identification based on morphology was carried out for only 62 individuals as these samples were useful enough to determine that on avocado and dandelion plants different thrips species are associated. Photographs of three species of thrips were taken with a motorized Leica M250A Stereo microscope using the plan Apo 1X objective and a DMC 4500 camera with AX support at 5X magnification.

DNA extraction. Thrips stored in alcohol were dried for 2 min on rice paper. Total DNA from thrips specimens was performed using a "salting-out" protocol adapted used by Rugman-Jones *et al.* (2006). Adults were transferred in a 1.5 ml Eppendorf tube with 100 µl of TNES buffer (50 mM Tris, pH 7.5, 400 mM NaCl, 20 mM EDTA, and 0.5 % SDS) and 1.7 µl of proteinase K (10 mg/ml). Each specimen received an abdominal puncture for DNA extraction. Furthermore, some adults were stored for morphological identification. Tubes were incubated at 37 °C for 18h. Afterward, proteins were precipitated with 28 µl of 5M NaCl for 15s with vigorous agitation. Proteins and adult specimens were sedimented on a microcentrifuge at 13.000 rpm for 5 min. The supernatant was transferred into a new 1.5 ml Eppendorf tube. DNA was precipitated from supernatant through the addition of ice-cold ethanol (100 %) and incubated for 1h at 20°C. DNA was further sedimented in a cold microcentrifuge at 4°C and 13.000 rpm for 5 min, ethanol was washed with ultra-pure sterile water-free with RNase and DNase and DNA was air-dried and finally dissolved in 30 µl of sterile water (Rugman-Jones *et al.* 2006).

COI gene and ITS region amplification. The barcode region COI-5' of 600 bp was made with primers LCO1490

and HCO2198 (Folmer *et al.* 1994) and amplified under the following conditions: 95°C (5 min), 35 cycles of 94 °C (30 s), 50.7 °C (45 s), 72 °C (1 min); and a final extension at 72 °C (10 min). The final 30 µl volume mix contained 1x PCR buffer, 1.5 mM MgCl₂, 0.2 mM dNTP, 0.06 U recombinant *Taq* polymerase, 0.4 µM de each primer, and 3 µL of DNA, complemented with ultra-pure water.

Amplification of the ITS region (1000-1200 bp) was performed with the 18SMP / 28SMP primers (De Grazia *et al.* 2016) and the polymerase chain reaction contained: 4 µl template; 3.0 µl of 10x PCR-buffer; 0.6 µl of 10 mM dNTP; 2.4 µl 25 mM MgCl₂; 0.48 µl of each primer; 0.1 µl of Thermo scientific™ recombinant *Taq* DNA polymerase and ultra-pure water to complete a final volume of 30 µl. Amplification was carried out in a T100™ Thermal Cycle Thermal Cycler (BIO RAD, CA, USA). DNA was denatured at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 45 s. Alignment was done at 53 °C for 45 s and an extension cycle at 72 °C for 2 min. A final extension cycle for 5 min was done at 72 °C to complete amplification. PCR products were visualized on 1.2 % agarose gels with Red gel® (Biotium, Hayward, CA, USA).

Amplified DNA fragments were sequenced (forward and reverse), assembled, aligned, and edited with CLC software v. 7.0.3 (QIAGEN, Hilden, Germany) and submitted to GenBank. Sequence codons were also checked with MEGA 7.0 (Kumar *et al.* 2016). All these sequences are available at the GenBank COI (MZ298133-MZ298225) and ITS (MZ331549-MZ331581).

Sequencing analysis phylogeny, barcoding gap, and haplotypes. All sequences obtained with both loci were compared to GenBank and BOLD databases using “BLASTn” (<http://www.ncbi.nlm.nih.gov/blast/>) and http://www.barcodinglife.org/index.php/IDS_OpenIdEngine, respectively (Supplementary Table 1). Two representatives per haplotype group were selected to perform the phylogeny of the COI gene. Sequences of the COI gene and ITS region were aligned using MUSCLE (Edgar, 2004). Two dendrograms were inferred separately for the COI gene and *ITS* region with the platform Beast 2.0 (Bouckaert *et al.* 2014). Data was analyzed in Beauti using a MCMC with 46 million generations. Each consensus tree obtained for the COI gene and *ITS* region was maintained every 4600 generations and preBurning was established for the first 10 million generations for the MCMC. The posterior probability was corroborated with Tracer v1.6 (Rambaut, 2014), the consensus trees were summarized with Treeannotator and graphed with Figtree (Rambaut, 2014).

The barcoding gap was calculated to determine the potential of the COI marker for the identification of the thrips species. For this, the ABGD (Automatic Barcode Gap Discovery) web software (Puillandre *et al.* 2012) was used with a K2P substitution model a maximum *a priori* intraspecific divergence value between 0.001 and 0.1, 20 recursive steps, and a relative gap width (X) of 3.0 as proposed by Iftikhar *et al.* (2016) for thrips.

An analysis of the genetic differences between the haplotypes of *F. gardeniae* and *F. gossypiana* was performed for COI gene and ITS region separately for both species, since

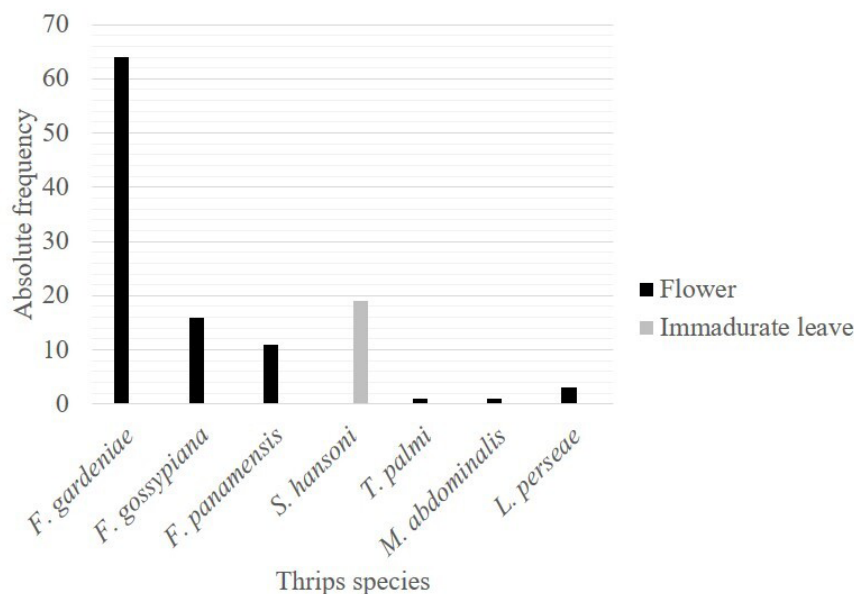


Figure 1. Percentage of the found thrips species, according to the different plant organs. Thrips were collected in avocado crops from Antioquia, North -West Colombia. Black = flower, grey = immature leaves.

phylogenetic analysis clustered both species within the same clade, suggesting no genetic differences between them. Seven additional COI GenBank sequences of Mexican origin were also used here. DnaSP v6 (Rozas *et al.* 2017) and Network v5 (Bandelt *et al.* 1999), were used to analyze COI and ITS haplotypes. Finally, an AMOVA (Analysis of Molecular Variance) using Arlequin 3.1 software (Excoffier *et al.* 2005) was performed to determine whether sampled individuals of the species *F. gardeniae* and *F. gossypiana* were genetically different according to F_{ST} estimations using COI sequences.

Results

Morphological identification carried out in 62/93 specimens (collected in avocado and *T. officinale* = dandelion) by Dr. Laurence Mound, demonstrated the presence of the following species in avocado and dandelion: *Frankliniella gardeniae* Moulton, *F. gossypiana* Hood, *F. panamensis* Hood, *Thrips trehernei* Priesner, *T. simplex* (Morison), *T. palmi* Karny, *Microcephalothrips abdominalis* (Crawford DL), *Scirtothrips hansonii* Mound & Hoddle, and *Liothrips perseae* Watson (Tubulifera). In general, the most abundant species found were *F. gardeniae* with 55.65 % followed by *S. hansonii* 16.52 % and *F. gossypiana* 13.91 %. However, *F. gardeniae* and *F. gossypiana* were found on flowers, whereas *S. hansonii* was found on leaves (Fig. 1). To our knowledge, this is the first time that *S. hansonii* has been reported on avocado in Colombia, while *M. abdominalis* and *F. panamensis* have not been reported on avocado before this study. *Thrips simplex* and *T. trehernei* were only found on dandelion flowers, suggesting that dandelion plant is not a reservoir of avocado thrips. They

were included in this study as many dandelion plants were surrounding the avocado trees and could serve as reservoirs of this insect.

The COI gene produced an amplification fragment of 600 bp in 93 individuals, but only 372/600 bp was used to perform the phylogenetic approximation. This was done to include another group of 137 sequences obtained from the GenBank to produce a Bayesian phylogeny (Fig. 2). Furthermore, the ITS sequences produced a 1242 bp segment from 61 samples that were also used to generate another Bayesian phylogeny (Fig. 3). The first dendrogram (Fig. 2) showed that the genus *Frankliniella* clustered all the species included here within this genus. The species *F. gardeniae* and *F. gossypiana* were clustered within the same group, demonstrating that no clear differentiation was obtained between these two species with this locus. *Frankliniella* was closely related to *Microcephalothrips*, which was only composed by the species *M. abdominalis*. Furthermore, the genus *Thrips* was also closely related to *Frankliniella* and *Microcephalothrips* and was composed by the species *T. palmi*, *T. simplex*, and *T. trehernei*. *Scirtothrips* were grouped separately from the other three genera in a group consisting of *S. hansonii*, *S. perseae*, and *S. dorsalis*. The topology obtained on this dendrogram suggests that more COI sequences are needed to improve the phylogeny of the genera *Microcephalothrips* and *Thrips*. In the second dendrogram (Fig. 3), the genus *Scirtothrips* was also separated from the genera *Frankliniella* and *Thrips*, corroborating the results obtained with the COI phylogeny. However, the close relationship found between these two genera demonstrates that more sequences are needed for the ITS region as grouping between these two genera did not differentiate the species well.

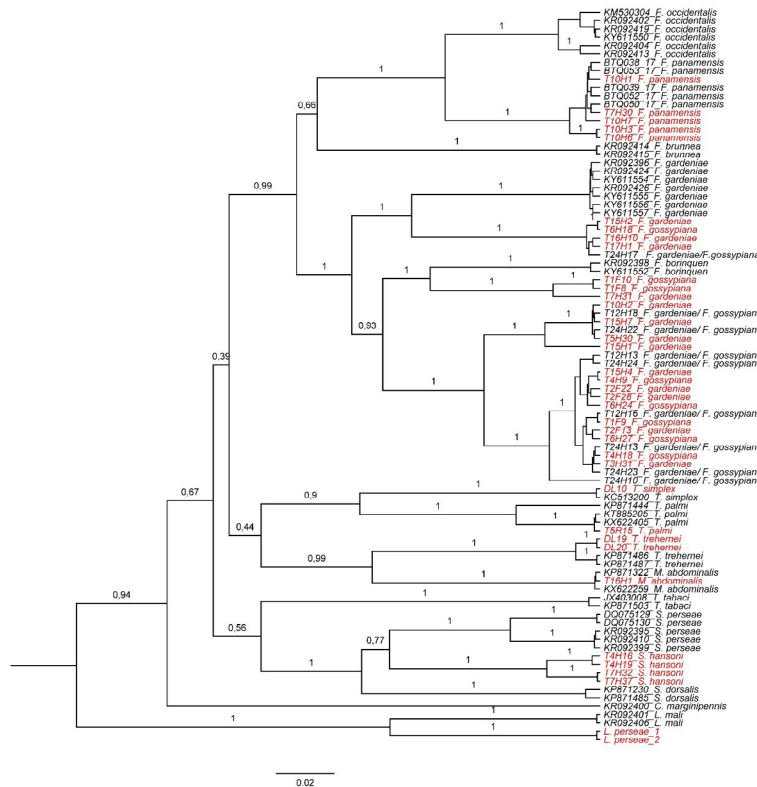


Figure 2. Bayesian phylogeny was obtained with COI sequences from avocado thrips. The sequences used as references are with their GenBank or Bold access codes. The thrips species found in this work were: *F. gardeniae*, *F. gossypiana*, *F. panamensis*, *T. palmi*, *T. simplex*, *T. trehernei*, *Microcephalothrips abdominalis*, and *S. hansonii*. Out-group = *L. perseae*. In red, in red, thrips samples identified morphologically and molecularly.

The ABGD barcoding gap analysis showed a genetic gap between 8 to 11 % (Fig. 4). The presence of 42 groups was observed which were composed of the different species of thrips (Supplementary Table 2) and in general, each group contained a single species. However, some species such as *F. panamensis* and *S. hansonii* formed several groups indicating intra-species variation. Additionally, in agreement with the other results obtained in phylogenetic analysis, genetic divergence between *F. gardeniae* and *F. gossypiana* was not evidenced, suggesting that these two species belong to one genetic unit.

Haplotype network revealed the existence of 19 haplotypes (Fig 5a), being H2 the most common. This haplotype had thirty-one individuals where four of them corresponded to *F. gossypiana*, followed by haplotypes H1, H6, H7, H8, and H3. The rest of the haplotypes presented a single individual, including *F. gardeniae* and *F. gossypiana*. Haplotype identification carried out with Dnasp showed that the species *F. gardeniae* and *F. gossypiana* cannot be differentiated with the COI marker. *Frankliniella gardeniae* have more haplotypes than *F. gossypiana* suggesting that the origin of the latter species is more recent. Additionally, Mexican *F. gardeniae* produced a single haplotype (H1) unlike the Colombian haplotypes that were divided into the remaining eighteen haplotypes. Mexican haplotypes were far apart from haplotypes of

the same genus from Colombia suggesting that *F. gardeniae* from Colombia is genetically distant from the same species from Mexico.

The ITS haplotype network (Fig. 5b) showed that some of the specimens that were collected on avocado might belong to the genus *Frankliniella* given their genetic proximity to sequences obtained from the GeneBank for the genus. Since fewer sequences were used here to analyze the genetics of this group, these haplotypes mainly corroborate the results obtained for the COI gene. In this case, no analysis of genetic differentiation was made for *F. gardeniae* and *F. gossypiana* since more information is needed in the databases of these two species. The haplotype network showed that the genus *Thrips* is closely related to *Frankliniella* as sequences of *T. simplex* and *T. sambuci* were close to *Frankliniella*. On the other hand, the genus *Scirtothrips* is not closely related to the other mentioned genera suggesting that is genetically different from them, corroborating the results obtained for the COI gene network and phylogeny.

Finally, AMOVA results obtained from COI sequences of *F. gardeniae* and *F. gossypiana* showed a low F_{ST} value that was not significant (Table 1), suggesting no genetic differentiation between these two species.

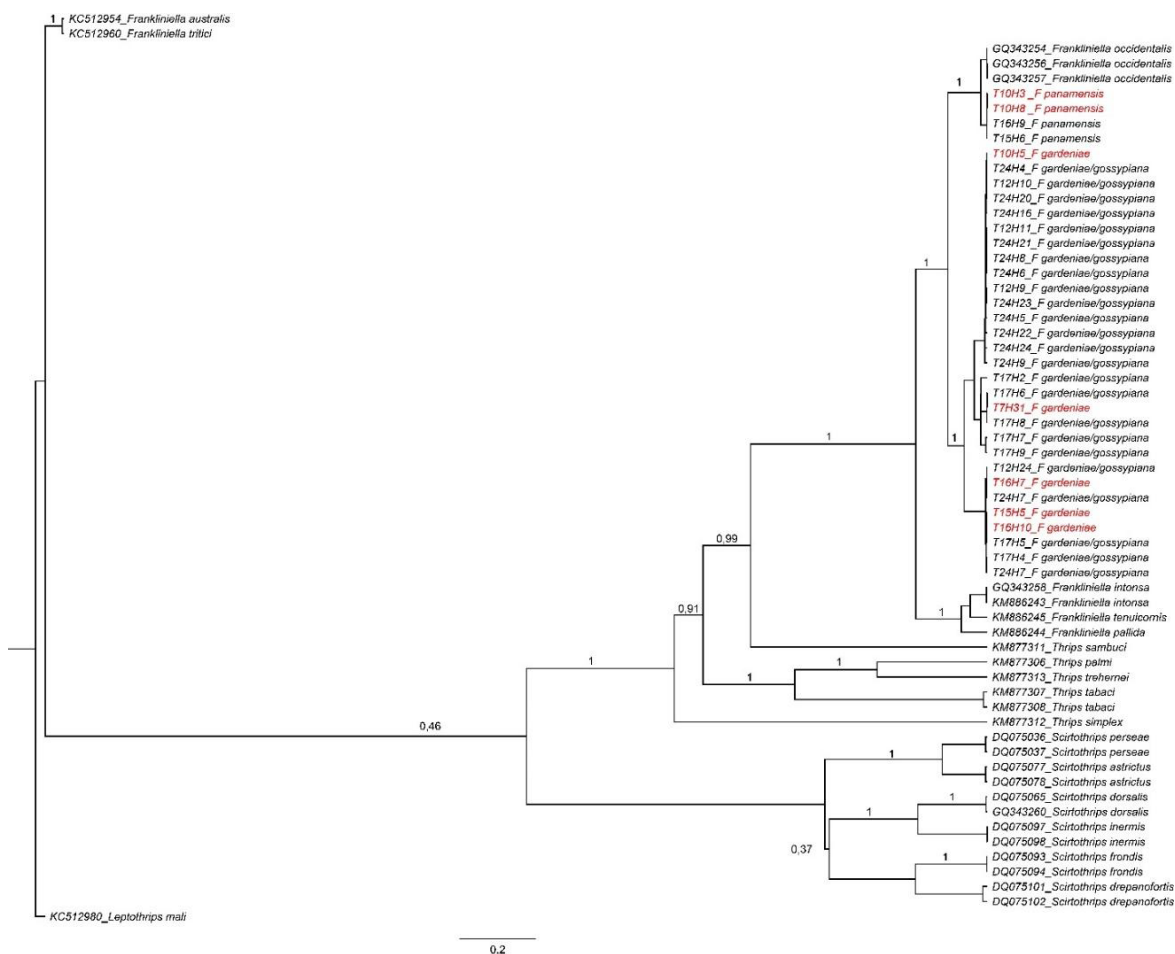


Figure 3. Bayesian phylogeny was obtained with ITS sequences from avocado thrips. The sequences modified as references are with their GenBank or Bold access codes. The thrips species found in this work were: *F. gardeniae*, *F. gossypiana*, *F. panamensis*, *T. palmi*, *T. simplex*, *T. trehernei*, *Microcephalothrips abdominalis*, and *S. hansonii*. Out-group = *L. mali*. In red, in red, thrips samples were identified morphologically and molecularly.

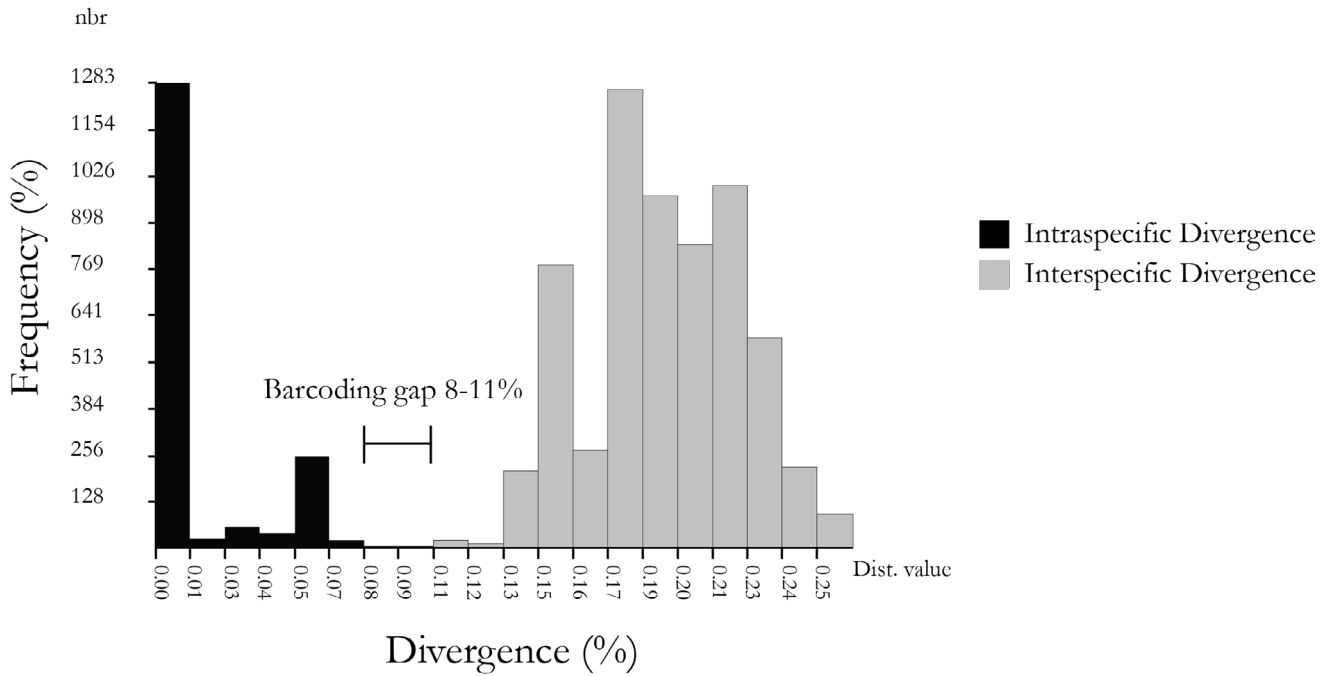


Figure 4. Analysis of the barcode gap of thrips species generated by automatic barcode gap detection (ABGD) (Puillandre *et al.* 2012). The histogram shows the K2P distance within and between each pair of samples of the COI gene.

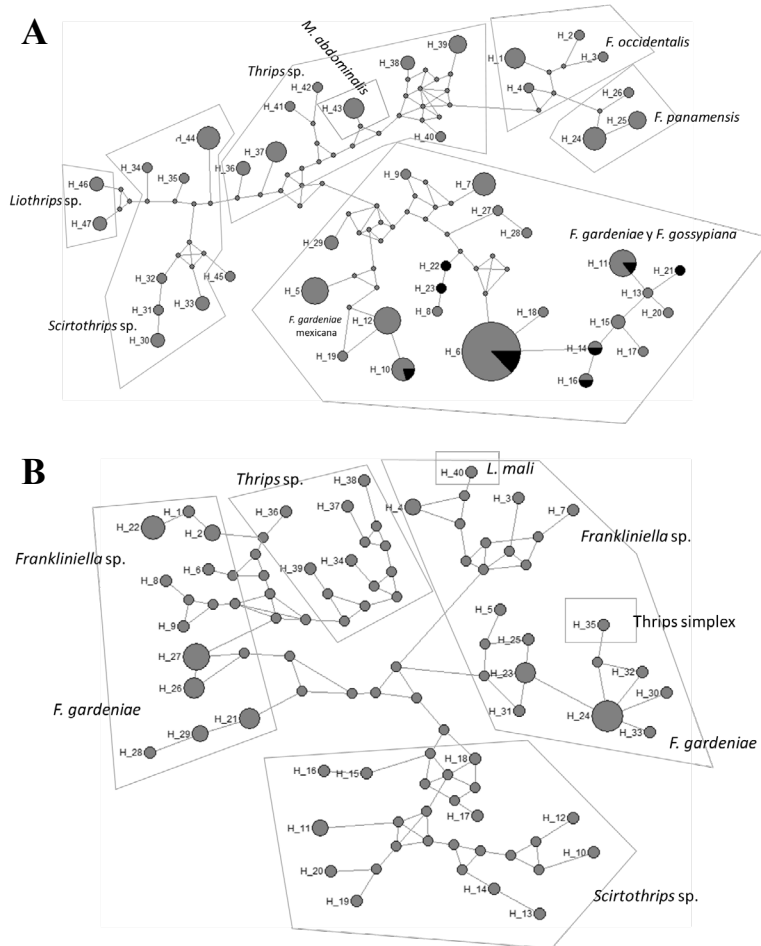


Figure 5. Haplotype networks. **a.** Haplotype network obtained for COI sequences from Antioquia avocado and GeneBank and BOLD Databases. **b.** Haplotype network obtained for ITS sequences from Antioquia avocado and GeneBank

Table 1. AMOVA results were obtained from genetic comparisons between individuals of the species *F. gardeniae* and *F. gossypiana* collected from avocado.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	F _{ST}	P
Among	1	18.7	0.1280 Va	7.60 %	0.00756	0.308
Within	69	1130.88	16.39 Vb	99.24 %		
Total	70	1149.6				

Discussion

So far, no molecular genetic studies of thrips have been done before this work in Colombia. DNA barcoding was employed here as it represents an effective method for species-level identification. Based on the molecular data obtained, we consider that *F. gardeniae* and *F. gossypiana* are likely to be the same species. However, to obtain more robust results it would be important to have more morphologically-identified specimens from both species to be studied at the molecular level. Bravo-Pérez *et al.* (2018) used sequences of COI to identify several thrips species on avocado from Mexico by using specimens collected from fruits and leaves. These authors mentioned that species identification was useful in differentiating *F. borinquen*, *F. brunnea*, *F. rostrata*, and *F. insularis* on avocado, demonstrating the efficacy of this gene for the identification of species belonging to the *Frankliniella* genus. A similar analysis, using the COI gene, was carried out by Brunner *et al.* (2004) with 10 thrips species. They found a high F_{ST} of 0.9713 between *Thrips angusticeps* and *T. tabaci* and low F_{ST} values such as 0.16 between *Echinothrips americanus* and *T. palmi*. The significant population differentiation between the 10 species involved showed that this gene successfully identified thrips species from different geographic origins (England, North America, Israel, amongst others).

When determining the percentage contribution of thrips species in crops per plant organ, *F. gardeniae*, *F. gossypiana*, and *F. panamensis* were found to be predominately associated with floral organs suggesting that this genus feed mainly on flowers. In contrast, *S. hansonii* was found mostly on young leaves. Mound and Teulon (1995) reported that members of the *Frankliniella* genus are mainly found on flowers and leaves, but *Scirtothrips* are present only on young leaves. In other studies, different thrips species have been reported to prefer different plant tissues. For example, *Heliethrips haemorrhoidalis* collected from citrus (Rutaceae) and *Quercus* (Fagaceae) prefer mostly old leaves (Marullo 2009). In a previous study conducted in Colombia on avocado, the following thrips species were found: *Frankliniella gardeniae* Moulton, *Karnyothrips* sp., *Leptothrips* sp., and *Selenothrips rubrocinctus* Giard, and on avocado (Echeverri-Florez *et al.* 2004). In our study, we found a higher number of thrips species but no potentially predatory thrips. Differences between these two studies carried out in Colombia might be due to the fact that our study was carried out in Antioquia, North-East Colombia whereas the study by Echeverri-Florez *et al.* (2004) was carried out in Risaralda and Caldas departments at the West-Center of Colombia. These differences may be due to environmental factors, management at the agrochemical level, or the presence of different adjacent crops. Another interesting aspect of our study is that the species found on dandelion:

Thrips simplex and *T. trehernei* differed from the species that attack avocado, discarding the possibility that dandelion could be a reservoir of thrips that affect avocado.

Sequencing of the barcode COI gene was used for the identification and elucidation of all the species collected in this study, and its implementation in avocado was very useful. Comparisons between the sequences obtained in this work together with those obtained from GenBank and BOLD databases showed matches with *F. panamensis*, *L. perseae*, *M. abdominalis*, *T. simplex*, *T. palmi*, and *T. trehernei*, with a high percentage of similarities (98 %). Similar results were obtained with ITS sequences, suggesting that both markers can also be implemented in the integrated pest management of this group of cryptic species of avocado. However, the COI gene allows obtaining a better image of the phylogenetic relationship of thrips species.

Phylogeny found for the nine species with COI gene and ITS region showed a close relation between *Frankliniella*, *Microcephalothrips*, and *Thrips* and a distant relation of these three genera with *Scirtothrips*. The topology of the phylogeny obtained with COI in this study coincided with previous studies (Iftikhar *et al.* 2016) and (Liu *et al.* 2017) showing that the genera *Scirtothrips* and *Thrips* are closely related and share a common ancestor with *Frankliniella*, corroborating the phylogenetic approach obtained here. Similarly, Rebijith *et al.* (2014) also found the same topology.

In the present study, only two loci were used to carry out a phylogeny and only members of the family Thripidae were analyzed. The results obtained support a close relation of the genera *Frankliniella*, *Microcephalothrips*, and *Thrips* with some differentiation with the genus *Scirtothrips*. However, the phylogenetic relationship between *F. gardeniae* and *F. gossypiana* remains difficult to determine. A recent phylogeny obtained by Bravo-Pérez *et al.* (2018) on avocado from Mexico found that *F. occidentalis* specimens might also constitute a group of cryptic species of avocado. In our study, *F. gardeniae* and *F. gossypiana* were found clustered within the same group, suggesting the possible existence of cryptic species or biotypes within the same species as they differ in morphology but did not differ in their genetics. To our knowledge, this is the first time that information on the genetic variation of these species is recorded at the genetic level in the world. AMOVA results also showed no genetic differentiation between *F. gardeniae* and *F. gossypiana* (F_{ST} = 0.0076, P > 0.05) supporting the hypothesis that these two species represent the same genetic unit, even though they differ morphologically.

In a population genetics study made with *Thrips tabaci*, a thrips species considered a relevant pest of onions in Australia, Westmore *et al.* (2013) showed that the species evolved into different biotypes, based on the analysis of differences of the COI gene (sequences) found between thrips sampling

sites (Westmore *et al.* 2013). Two biotypes were found, one mainly associated with onion which does not transmit viruses, and the other with tobacco which transmits viruses, differing only in 6 nucleotide positions. In our case, *F. gardeniae* and *F. gossypiana* differed in 41 nucleotide sites meaning that *F. gossypiana* might represent a biotype or a subspecies of *F. gardeniae*. However, according to Hebert *et al.* (2003), if two populations differ from 2 % K2 distances, they represent different species, and the results obtained here for *F. gardeniae* and *F. gossypiana* showed K2 = 6 % (data not shown).

Classical taxonomy using morphological characters cannot always be employed in the identification of thrips species in all life stages, as phenotypic variation can be insufficient (Brunner *et al.* 2004; Rebijith *et al.* 2014). The presence of unusual morphological forms on different hosts, small size, co-existence on the same host, complex life cycles, parthenogenetic lifestyle, amongst other traits, makes difficult the identification of species in this group (Rebijith *et al.* 2014; Iftikhar *et al.* 2016). As an alternative, the proposed concept of DNA barcoding could be a powerful tool to identify all metazoan species employing 658 bp fragment of the 5' end of the mitochondrial COI locus (Hajibabaei *et al.* 2007). DNA barcoding has been employed for discriminating cryptic species, biotypes, haplotypes, and host-associated pests. Therefore, sequencing this gene plays an important role for integrated pest management programs since it will allow to easily identify the species occurring in the crop thus generating better monitoring methods. Having an easy to implement identification method will help to identify the preferred areas of the insect on the plant as well as other possible hosts (Hajibabaei *et al.* 2007; Rebijith *et al.* 2014; Iftikhar *et al.* 2016; Liu *et al.* 2017). Implementing the use of sequences from the ITS region also might be useful to corroborate the identification of thrips in avocado and other crops. However, in our case, the ITS region was difficult to amplify and therefore it was not possible to have an adequate number of sequences. This might have been due to the quality and quantity of the DNA obtained from thrips, and to the fact that the primers used were generic for the genus. This difficulty was particularly seen with *S. hansonii* which is one of the smallest insects, so it may be related to the amount of hemolymph obtained from it. The fact that the COI gene amplified better might be due to better annealing of primers or because it is a mitochondrial gene, it might be present in higher copy numbers in the insect cells.

Conclusions

The use of the COI gene and ITS region allowed for the identification of avocado thrips species. In addition, a good hypothesis of the phylogenetic relationship of these species using the COI gene was obtained. Molecular analysis results suggest that *F. gardeniae* and *F. gossypiana* are the same genetic unit and that *F. gardeniae* from Colombia is genetically distant from the same species from Mexico. However, we obtained few sequences from the ITS region, perhaps due to the quantity and quality of the DNA extracted from thrips or because of the primers used in this work. Therefore, we suggest that future studies should use another extraction method or other set of primers better suited for the sampled species. This study showed that DNA sequencing is a fast and effective method to identify most thrips species in avocado and it could be implemented in integrated pest management programs of this group of insects in Colombia. Identification of pest species is

relevant for the improvement of their control given that specific pesticides should be used for each species and in the case of avocado at least seven cryptic species of thrips were found in this study.

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Author's contributions

Daniela Cano-Calle participated in experimentation processing, design, data processing, analysis, and discussion of the information in the manuscript and the writing of the manuscript.

Clara Inés Sadamando-Benjumea participated in the analysis, data processing, discussion of the information and the writing of the manuscript.

Claudia Ximena Moreno-Herrera participated in the design, project administration, discussion, supervision, review, and editing of the manuscript.

Rafael Eduardo Arango-Isaza participated in the design, data processing, analysis, and discussion of the information in the manuscript and the writing of the manuscript.

Supplementary Table 1. List of specimens sequenced for phylogenetic analysis with Cytochrome Oxidase I (COI)

Code	Species	Farm	Host plant	Municipality	Coordinates
DL10	<i>T. simplex</i>	Fruty green	Dandelion	El Retiro	06°03'00"N, 75°30'00"W
DL13	<i>T. trehernei</i>	Fruty green	Dandelion	El Retiro	06°03'00"N, 75°30'00"W
DL19	<i>T. trehernei</i>	Fruty green	Dandelion	El Retiro	06°03'00"N, 75°30'00"W
DL20	<i>T. trehernei</i>	Fruty green	Dandelion	El Retiro	06°03'00"N, 75°30'00"W
DL8	<i>T. trehernei</i>	Fruty green	Dandelion	El Retiro	06°03'00"N, 75°30'00"W
T1F3	<i>F. panamensis</i>	Fruty green	Avocado	El Retiro	06°03'00"N, 75°30'00"W
T1F5	<i>F. panamensis</i>	Fruty green	Avocado	El Retiro	06°03'00"N, 75°30'00"W
T1F8	<i>F. gossypiana</i>	Fruty green	Avocado	El Retiro	06°03'00"N, 75°30'00"W
T1F9	<i>F. gossypiana</i>	Fruty green	Avocado	El Retiro	06°03'00"N, 75°30'00"W
T1F10	<i>F. gossypiana</i>	Fruty green	Avocado	El Retiro	06°03'00"N, 75°30'00"W
T2F13	<i>F. gardeniae</i>	La María	Avocado	San Vicente	06°16'37"N, 75°23'01"W
T2F23	<i>F. gardeniae</i>	La María	Avocado	San Vicente	06°16'37"N, 75°23'01"W
T2F22	<i>F. gardeniae</i>	La María	Avocado	San Vicente	06°16'37"N, 75°23'01"W
T2F28	<i>F. gardeniae</i>	La María	Avocado	San Vicente	06°16'37"N, 75°23'01"W
T2F9	<i>F. gardeniae</i>	La María	Avocado	San Vicente	06°16'37"N, 75°23'01"W
T3H1	<i>F. gardeniae</i>	Fruty green	Avocado	El Retiro	06°03'00"N, 75°30'00"W
T3H19	<i>F. gardeniae</i>	Fruty green	Avocado	El Retiro	06°03'00"N, 75°30'00"W
T3H26	<i>F. gardeniae</i>	Fruty green	Avocado	El Retiro	06°03'00"N, 75°30'00"W
T3H30	<i>F. gardeniae</i>	Fruty green	Avocado	El Retiro	06°03'00"N, 75°30'00"W
T3H31	<i>F. gardeniae</i>	Fruty green	Avocado	El Retiro	06°03'00"N, 75°30'00"W
T4H9	<i>F. gossypiana</i>	La María	Avocado	San Vicente	06°16'37"N, 75°23'01"W
T4H16	<i>S. hansonii</i>	La María	Avocado	San Vicente	06°16'37"N, 75°23'01"W
T4H18	<i>F. gossypiana</i>	La María	Avocado	San Vicente	06°16'37"N, 75°23'01"W
T4H19	<i>S. hansonii</i>	La María	Avocado	San Vicente	06°16'37"N, 75°23'01"W
T5H30	<i>F. gardeniae</i>	La Felisa	Avocado	Marinilla	06°08'01"N, 75°20'01"W
T5R15	<i>T. palmi</i>	La Felisa	Avocado	Marinilla	06°08'01"N, 75°20'01"W
T5R20	<i>F. gossypiana</i>	La Felisa	Avocado	Marinilla	06°08'01"N, 75°20'01"W
T6H18	<i>F. gossypiana</i>	Aquella	Avocado	El Carmen de Viboral	06°03'46"N, 75°20'54"W
T6H21	<i>F. gossypiana</i>	Aquella	Avocado	El Carmen de Viboral	06°03'46"N, 75°20'54"W
T6H24	<i>F. gossypiana</i>	Aquella	Avocado	El Carmen de Viboral	06°03'46"N, 75°20'54"W
T6H27	<i>F. gossypiana</i>	Aquella	Avocado	El Carmen de Viboral	06°03'46"N, 75°20'54"W
T6R29	<i>F. gossypiana</i>	Aquella	Avocado	El Carmen de Viboral	06°03'46"N, 75°20'54"W
T7H30	<i>F. panamensis</i>	Fruty green	Avocado	El Retiro	06°03'00"N, 75°30'00"W
T7H31	<i>F. gardeniae</i>	Fruty green	Avocado	El Retiro	06°03'00"N, 75°30'00"W
T7H32	<i>S. hansonii</i>	Fruty green	Avocado	El Retiro	06°03'00"N, 75°30'00"W
T7H33	<i>F. gardeniae</i>	Fruty green	Avocado	El Retiro	06°03'00"N, 75°30'00"W
T7H36	<i>F. gardeniae</i>	Fruty green	Avocado	El Retiro	06°03'00"N, 75°30'00"W
T7H37	<i>S. hansonii</i>	Fruty green	Avocado	El Retiro	06°03'00"N, 75°30'00"W
T10H1	<i>F. panamensis</i>	La Vega	Avocado	La Ceja	06°03'32"N, 75°24'50"W

T10H2	<i>F. gardeniae</i>	La Vega	Avocado	La Ceja	06°03'32"N, 75°24'50"W
T10H3	<i>F. panamensis</i>	La Vega	Avocado	La Ceja	06°03'32"N, 75°24'50"W
T10H5	<i>F. gardeniae</i>	La Vega	Avocado	La Ceja	06°03'32"N, 75°24'50"W
T10H6	<i>F. panamensis</i>	La Vega	Avocado	La Ceja	06°03'32"N, 75°24'50"W
T10H7	<i>F. panamensis</i>	La Vega	Avocado	La Ceja	06°03'32"N, 75°24'50"W
T10H8	<i>F. panamensis</i>	La Vega	Avocado	La Ceja	06°03'32"N, 75°24'50"W
T12H3	NA	Los Rincones	Avocado	Rionegro	06°05'00"N, 75°23'34"W
T12H9	NA	Los Rincones	Avocado	Rionegro	06°05'00"N, 75°23'34"W
T12H10	NA	Los Rincones	Avocado	Rionegro	06°05'00"N, 75°23'34"W
T12H11	NA	Los Rincones	Avocado	Rionegro	06°05'00"N, 75°23'34"W
T12H13	NA	Los Rincones	Avocado	Rionegro	06°05'00"N, 75°23'34"W
T12H16	NA	Los Rincones	Avocado	Rionegro	06°05'00"N, 75°23'34"W
T12H17	NA	Los Rincones	Avocado	Rionegro	06°05'00"N, 75°23'34"W
T12H18	NA	Los Rincones	Avocado	Rionegro	06°05'00"N, 75°23'34"W
T12H24	NA	Los Rincones	Avocado	Rionegro	06°05'00"N, 75°23'34"W
T15H1	<i>F. gardeniae</i>	La escondida	Avocado	Rionegro	06°08'00"N, 75°25'00"W
T15H10	<i>F. gardeniae</i>	La escondida	Avocado	Rionegro	06°08'00"N, 75°25'00"W
T15H2	<i>F. gardeniae</i>	La escondida	Avocado	Rionegro	06°08'00"N, 75°25'00"W
T15H3	<i>F. panamensis</i>	La Escondida	Avocado	Rionegro	06°08'00"N, 75°25'00"W
T15H5	<i>F. gardeniae</i>	La Escondida	Avocado	Rionegro	06°08'00"N, 75°25'00"W
T15H7	<i>F. gardeniae</i>	La Escondida	Avocado	Rionegro	06°08'00"N, 75°25'00"W
T15H8	<i>F. gardeniae</i>	La Escondida	Avocado	Rionegro	06°08'00"N, 75°25'00"W
T15H9	<i>F. gardeniae</i>	La Escondida	Avocado	Rionegro	06°08'00"N, 75°25'00"W
T16H1	<i>Microcephalothrips abdominalis</i>	El Imperio	Avocado	Amaga	NA
T16H10	<i>F. gardeniae</i>	El Imperio	Avocado	Amaga	NA
T16H3	<i>F. gardeniae</i>	El Imperio	Avocado	Amaga	NA
T16H4	<i>F. gardeniae</i>	El Imperio	Avocado	Amaga	NA
T16H7	<i>F. gardeniae</i>	El Imperio	Avocado	Amaga	NA
T17H1	<i>F. gardeniae</i>	Bella Vista	Avocado	Jardin	NA
T24H1	NA	Varahonda	Avocado	Rionegro	06°05'09"N, 75°26'40"W
T24H2	NA	Varahonda	Avocado	Rionegro	06°05'09"N, 75°26'40"W
T24H3	NA	Varahonda	Avocado	Rionegro	06°05'09"N, 75°26'40"W
T24H5	NA	Varahonda	Avocado	Rionegro	06°05'09"N, 75°26'40"W
T24H6	NA	Varahonda	Avocado	Rionegro	06°05'09"N, 75°26'40"W
T24H8	NA	Varahonda	Avocado	Rionegro	06°05'09"N, 75°26'40"W
T24H7	NA	Varahonda	Avocado	Rionegro	06°05'09"N, 75°26'40"W
T24H9	NA	Varahonda	Avocado	Rionegro	06°05'09"N, 75°26'40"W
T24H10	NA	Varahonda	Avocado	Rionegro	06°05'09"N, 75°26'40"W
T24H11	NA	Varahonda	Avocado	Rionegro	06°05'09"N, 75°26'40"W
T24H12	NA	Varahonda	Avocado	Rionegro	06°05'09"N, 75°26'40"W
T24H13	NA	Varahonda	Avocado	Rionegro	06°05'09"N, 75°26'40"W
T24H14	NA	Varahonda	Avocado	Rionegro	06°05'09"N, 75°26'40"W
T24H16	NA	Varahonda	Avocado	Rionegro	06°05'09"N, 75°26'40"W

T24H18	NA	Varahonda	Avocado	Rionegro	06°05'09"N, 75°26'40"W
T24H17	NA	Varahonda	Avocado	Rionegro	06°05'09"N, 75°26'40"W
T24H19	NA	Varahonda	Avocado	Rionegro	06°05'09"N, 75°26'40"W
T24H20	NA	Varahonda	Avocado	Rionegro	06°05'09"N, 75°26'40"W
T24H21	NA	Varahonda	Avocado	Rionegro	06°05'09"N, 75°26'40"W
T24H22	NA	Varahonda	Avocado	Rionegro	06°05'09"N, 75°26'40"W
T24H23	NA	Varahonda	Avocado	Rionegro	06°05'09"N, 75°26'40"W
T24H24	NA	Varahonda	Avocado	Rionegro	06°05'09"N, 75°26'40"W
TUB1 (T11H1)	<i>Liothrips perseae</i>	La Paz	Avocado	El Retiro	05°59'18"N, 75°30'32"W
TUB2 (T7H2)	<i>Liothrips perseae</i>	La Vega	Avocado	La Ceja	06°3'31,6"N, 75°24'50"W

NA: no information is given

Supplementary Table 2. List of groups generated by barcoding gap analysis with ABGD web program

Group	n	Specie(s)
Group 1	31	T3H31 <i>F. gardeniae</i> T7H33 <i>F. gardeniae</i> T7H36 <i>F. gardeniae</i> T10H5 <i>F. gardeniae</i> T15H9 <i>F. gardeniae</i> T3H26 <i>F. gardeniae</i> T3H19 <i>F. gardeniae</i> T3H1 <i>F. gardeniae</i> T2F23 <i>F. gardeniae</i> T2F9 <i>F. gardeniae</i> T12H3 T12H10 T12H11 T12H17 T24H1 T24H2 T24H3 T24H5 T24H6 T24H9 T24H11 T24H12 T24H13 T24H14 T24H16 T24H18 T24H20 T6R29 <i>F. gossypiana</i> T6H21 <i>F. gossypiana</i> T5R20 <i>F. gossypiana</i> T4H18 <i>F. gossypiana</i>
Group 2	3	T7H31 <i>F. gardeniae</i> T1F10 <i>F. gossypiana</i> T1F8 <i>F. gossypiana</i>
Group 3	3	T15H2 <i>F. gardeniae</i> T15H8 <i>F. gardeniae</i> T12H24
Group 4	5	T10H1 <i>F. panamensis</i> T7H30 <i>F. panamensis</i> T15H3 <i>F. panamensis</i> T1F5 <i>F. panamensis</i> T1F3 <i>F. panamensis</i>
Group 5	1	<i>Frankliniella tritici</i> KC513153
Group 6	1	<i>Frankliniella tritici</i> KM532368
Group 7	1	<i>Frankliniella fortissima</i> KT935672
Group 8	2	<i>Frankliniella occidentalis</i> MF750672 <i>Frankliniella occidentalis</i> KM530304
Group 9	4	T7H32 <i>S. hansonii</i> T7H37 <i>S. hansonii</i> T4H19 <i>S. hansonii</i> T4H16 <i>S. hansonii</i>
Group 10	1	<i>Scirtothrips astrictus</i> EU100996
Group 11	2	<i>Scirtothrips perseae</i> DQ075129 <i>Scirtothrips perseae</i> DQ075130
Group 12	2	<i>Scirtothrips dorsalis</i> KP871485 <i>Scirtothrips dorsalis</i> KP871230
Group 13	2	<i>Scirtothrips oligochaetus</i> KP871302 <i>Scirtothrips oligochaetus</i> EU100992
Group 14	2	<i>Thrips trehernei</i> KP871487 <i>Thrips trehernei</i> KP871486
Group 15	2	<i>Thrips simplex</i> KC513200 DL10 <i>T. simplex</i>
Group 16	4	<i>Thrips palmi</i> KT885205 <i>Thrips palmi</i> KX622405 <i>Thrips palmi</i> KP871444 T5R15 <i>T. palmi</i>
Group 17	2	<i>Thrips tabaci</i> JX403008 <i>Thrips tabaci</i> KP871503
Group 18	2	<i>Thrips flavus</i> KP871501 <i>Thrips flavus</i> KP871500
Group 19	2	<i>Thrips coloratus</i> KP871489 <i>Thrips coloratus</i> KP871405
Group 20	2	<i>Thrips apicatus</i> KP871469 <i>Thrips apicatus</i> KP871439
Group 21	2	<i>Thrips hawaiiensis</i> KP871233 <i>Thrips hawaiiensis</i> KP871187
Group 22	2	<i>Thrips vulgatissimus</i> KM537771 <i>Thrips vulgatissimus</i> KM535536
Group 23	4	T16H1 <i>M. abdominalis</i> <i>Microcephalothrips abdominalis</i> KX622259 <i>Microcephalothrips abdominalis</i> KP871322 <i>Microcephalothrips abdominalis</i> KX622258
Group 24	5	T5H30 <i>F. gardeniae</i> T10H2 <i>F. gardeniae</i> T15H7 <i>F. gardeniae</i> T12H18 T24H22
Group 25	1	T15H1 <i>F. gardeniae</i>
Group 26	7	T15H4 <i>F. gardeniae</i> T3H30 <i>F. gardeniae</i> T12H9 T24H8 T24H19 T24H21 T4H9 <i>F. gossypiana</i>
Group 27	1	T2F28 <i>F. gardeniae</i>

Group 28	2	T2F13_ <i>F_gardeniae</i> T6H27_ <i>F_gossypiana</i>
Group 29	2	T12H13 T24H24
Group 30	2	T12H16 T1F9_ <i>F_gossypiana</i>
Group 31	1	T24H10
Group 32	1	T24H23
Group 33	1	T2F22_ <i>F_gardeniae</i>
Group 34	1	T6H24_ <i>F_gossypiana</i>
Group 35	7	T15H5_ <i>F_gardeniae</i> T15H10_ <i>F_gardeniae</i> T16H3_ <i>F_gardeniae</i> T16H4_ <i>F_gardeniae</i> T16H7_ <i>F_gardeniae</i> T16H10_ <i>F_gardeniae</i> T17H1_ <i>F_gardeniae</i>
Group 36	1	T24H7
Group 37	1	T24H17
Group 38	1	T6H18_ <i>F_gossypiana</i>
Group 39	3	T10H3_ <i>F_panamensis</i> T10H6_ <i>F_panamensis</i> T10H8_ <i>F_panamensis</i>
Group 40	1	T10H7_ <i>F_panamensis</i>
Group 41	5	DL20_ <i>T_trehernei</i> DL19_ <i>T_trehernei</i> DL13_ <i>T_trehernei</i> <i>Thrips_sp_KM535375</i> <i>Thrips_sp_KM537823</i>
Group 42	1	DL8_ <i>T_trehernei</i>